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RESPIRATORY MOVEMENTS AND RAPID EYE MOVEMENT SLEEP IN THE FOETAL LAMB

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SUMMARY

1. In foetal lambs from 40 days gestation (0·27 of term) onwards delivered into a warm saline bath, apparently spontaneous breathing movements were present intermittently. They became deeper and more rapid with increasing age.

2. In foetal lambs (from 0·66 of term) in which observations were made for many days after chronic implantation of tracheal, carotid and amniotic catheters, rapid irregular respiratory movements were present up to 40% of the time, and brief gasps also were seen.

3. The presence of these movements was unrelated to the foetal carotid blood gas values over a wide range of spontaneous variation.

4. These foetal breathing movements were accompanied by comparatively small alterations of pulmonary volume recorded from a tracheal flowmeter, insufficient to clear the tracheal dead space. Occasionally a more prolonged expiration led to the outward flow of fluid.

5. A description is given of sleep and wakefulness in foetal lambs from 0·78 of term.

6. Rapid irregular breathing was associated with rapid eye movement sleep as seen in a warm saline bath or, *in utero*, as inferred from records of eye movements and electrocortical activity.

7. Respiratory movements were often associated with relatively large variations in foetal heart rate, blood pressure and descending aortic blood flow.

8. Rapid irregular foetal breathing was unaffected by section or blockade of the cervical vagi, but was abolished by general anaesthesia.

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9. It is concluded that respiratory movements are normally but intermittently present in the foetal lamb over the greater part of gestation.

INTRODUCTION

Though there have been reports of foetal breathing *in utero* for many years (Preyer, 1885), there has been doubt as to what extent this phenomenon is spontaneous, or dependent upon the conditions of the experiment, which may involve tactile, thermal or other sensory stimuli or a departure from normal blood gas values (e.g. Windle, 1941). Yet mature infants of mammalian species are remarkably competent at maintaining prolonged hyperpnoea after birth; this suggests that they may well have used their respiratory muscles from time to time *in utero*, an hypothesis which it was proposed to test by implanting a transducer or catheters in the foetus (Dawes, 1968).

Barcroft & Barron (1937*a*, *b*) and Barcroft (1946) reported experiments on foetal lambs observed under maternal spinal or general anaesthesia, either in the amniotic sac or on delivery into a warm saline bath. They described movements of the respiratory musculature from 40 to 60 days gestational age (term is ~ 147 days). The movements were not continuous, were initiated by stimulation of the foetus (e.g. touching the face) and the account suggests that such movements were not normally present, but resulted from sensory stimuli. After 60 days gestation respiratory movements were difficult to elicit. It was decided to begin by repeating these observations, over the period 40–100 days gestation, using manometers (of a type not available to Barcroft & Barron in 1937) in order to distinguish inspiratory efforts from other foetal movements.

The results show that respiratory movements are present, apparently spontaneously, in foetal lambs from 40 days gestation onwards. They have also been detected by implantation of tracheal catheters and flowmeters in lambs from 92 days gestation onwards and, towards term, their appearance is associated with the phenomena of rapid-eye-movement sleep. A brief account of these observations has been published (Dawes, Fox, Leduc, Liggins & Richards, 1970).

METHODS

Observations were made on 77 cross-bred (Cheviot-Border Leicester) foetal lambs during acute or chronic experiments.

Acute experiments. Foetal lambs of 40–142 days gestation were delivered into a warm (39–40° C) saline (0·9 % (w/v) NaCl) bath under maternal epidural or spinal anaesthesia. The foetus was wholly immersed; the umbilical cord was intact. In lambs of < 55 days gestation age intrapulmonary pressure was recorded from a catheter introduced through the chest wall into a pleural space or lung; in older lambs it was recorded from a catheter introduced via the trachea and/or oesophagus.

Chronic experiments. The ewes, 92–138 days pregnant, were anaesthetized by injection of 10 ml. of a mixture of bupivicaine (0·25 %, w/v) and lignocaine (1 %, w/v) into the lumbar epidural space. The abdominal skin was shorn and disinfected, with the ewe supine. The uterus was exposed through a mid line incision below the navel. The foetal head was held with the neck extended, within the upper part of the uterine horn, and an incision was made through the uterus, membranes and the skin of the neck about 3 cm long in the mid line below the larynx (Fig. 1). The edges of the uterine wall and foetal skin were united with forceps to minimize the loss of amniotic fluid. A carotid artery was catheterized with a polyethylene tube (i.d. 0·86 mm, o.d. 1·27 mm) sheathed within a polyvinyl tube save for that portion

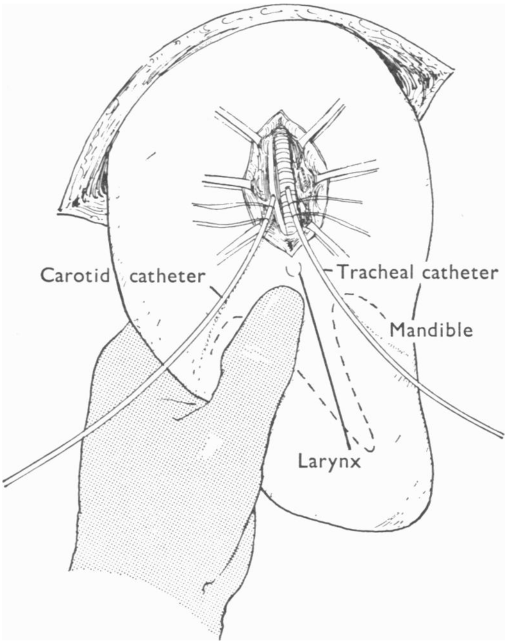

Fig. 1. Operative exposure of the foetal neck.

which was inserted within the vascular lumen. A similar catheter was passed into the trachea through a small puncture made with the tip of a hypodermic needle, and was advanced 7·5 cm to lie within the thoracic inlet. An open-ended catheter was always placed in the amniotic cavity. In two lambs an open-ended fluid-filled catheter was inserted into the oesophagus through a small incision through its wall, and passed down into the thorax. In five lambs the trachea was partially divided just below the larynx (above the point of entry of the tracheal catheter), a cannulated electromagnetic flowmeter of size such that it completely filled the lumen (8·9 mm o.d., 4–5 mm i.d.; Clark & Wyatt, 1969) was inserted and the cut ends were united with sutures. Both vagi were divided and short sections were removed in the upper cervical region (two lambs) or the intact vagi had fine catheters placed alongside them so that the nerves could be blocked temporarily by injection of a local anaesthetic (two lambs).

A triple antibiotic spray containing polymyxin B, bacitracin and neomycin (Framyspray, Fisons Pharmaceuticals Ltd) was applied to the foetal incisions before closure of the skin. Penicillin (100,000 units in 10 ml. saline) was injected through the amniotic catheter when the operation was completed.

The foetal electrocorticogram was recorded from electrodes implanted biparietally. A mid line incision was made through the scalp from the mid-orbital line to the occiput. The periosteum was scraped off the calvarium. Stainless-steel screws were inserted through burr-holes over the central parietal cortex, 9 mm to either side of the mid line; their ends rested on the dura mater. They were attached to a cable whose screen was embedded in the subcutaneous tissues. A mould was formed from dental acrylic cement over the electrodes, to provide mechanical stability and insulation from the scalp and amniotic fluid.

To record foetal eye movements the superior orbital ridge was exposed on one side. Stainless-steel screw electrodes were inserted through burr-holes 2·5 mm into the bone, 1 cm apart along the ridge, and were encased in dental acrylic cement. Thus the electrodes lay obliquely so as to detect either vertical or horizontal eye movements from the change in orientation of the corneo-retinal potential. Descending aortic flow was measured in three lambs with a cuff electromagnetic flowmeter probe 8 mm i.d., implanted retroperitoneally below the origin of the renal arteries, using a recording system of high fidelity (Clark & Wyatt, 1969).

A polyvinyl tube of 2 mm i.d. was inserted into the ewe's carotid artery for 28 cm. The proximal end was secured to the skin of the back, where it was accessible without disturbing the animal. Likewise the foetal and amniotic catheters, electrode and flowmeter leads were sewn to the skin of the flank where they emerged from the abdominal cavity.

The ewes were kept in metabolic cages after the operation and were fed hay and a mixture of oats and soya meal *ad libitum*. A single dose of 500,000 units of penicillin was given intramuscularly. The foetal carotid, tracheal and amniotic catheters were connected to pressure transducers (Devices). The arterial catheters were perfused continuously at 4·8 ml./24 hr with a saline solution (NaCl 0·9 % w/v) containing heparin (0·1 mg/ml.) and penicillin (1000 u./ml.).

Recording methods. Foetal carotid arterial pressure, tracheal and oesophageal pressures, amniotic pressure, tracheal and aortic flows, the electrocortical and transorbital potentials were recorded on a Schwarzer polygraph. The tracheal gauge-head and catheter had a natural frequency of at least 6 Hz and was heavily damped. The foetal heart rate was recorded from a rapid response ratemeter (Wyatt, 1957) or instantaneous ratemeter (Devices, 2751) actuated from the carotid pressure pulse. The foetal tracheal volume displacement was recorded from the volume flow with an integrating circuit using time constants of 0·1–0·5 sec, whose performance was verified by graphical analysis. The electrocortical and transorbital potential recorders had a response which was flat from 0·5 to 50 Hz. Electrocortical records were made with recording speeds of 9 mm/min or 30 mm/sec (the latter for at least 45 min twice a day up to 8 days).

Blood samples (each of 0·6–0·8 ml.) were taken into heparinized glass syringes from the foetal and maternal carotid arteries. The samples were analysed at once for pH, P_{O_2}, and P_{CO_2} using Radiometer electrodes, and the results were corrected for the difference between electrode and maternal temperature. In chronic experiments, the likelihood of introducing infection into the foetuses during blood sampling was minimized by covering the outlets of the stop-cocks with rubber caps that were soaked in tincture of hibitane and were replaced after sampling.

The supine position

The supine position (in preference to a lateral position) was used for the 2–3 hr required to implant catheters and flow transducers, under epidural anaesthesia, because surgical access was easier. The ewes lay quietly, often apparently asleep, and there was no evidence of sudden circulatory collapse such as often happens with women placed in a supine position in late pregnancy. Some concern was felt about maternal gas exchange because of the alteration in the relative positions of the heart and lungs in the vertical axis, and the possible effect on ventilation-perfusion ratios.

Observations were made on ten additional ewes, five under epidural and five under light chloralose (20–30 mg/kg) anaesthesia. Comparisons of arterial blood gas values were made during periods of half an hour in the right lateral or supine positions, each set of observations being bracketed (e.g. lateral, supine, lateral). Under epidural anaesthesia in the supine position, the ewe's arterial P_{O_2} was satisfactorily high (mean P_{O_2} 94·9 ± 1·9 mm Hg, P_{CO_2} 37·9 ± 1·0 mm Hg, pH 7·38 ± 0·01), significantly higher than in the lateral position by a mean 6·2 mm Hg on paired comparisons. The differences in arterial P_{CO_2} and pH were statistically insignificant. The improvement in arterial P_{O_2} with the supine position was also present under light general anaesthesia in four of five ewes. In two ewes the alveolar ventilation was measured; it was greater in the supine position by 19–32 %, partly because of a small increase in respiratory rate. The explanation of this has not been explored further. There was no significant change in arterial pressure with position, when allowance was made for the variation in the height of the heart. The supine position is evidently satisfactory for surgery in adult pregnant sheep.

RESULTS

Delivery into a warm saline bath: immature lambs

Observations were made on twenty-two foetal lambs delivered from twelve ewes at 40–55 days gestation age (4·9–42·5 g body weight), and on twenty-one foetal lambs delivered from fifteen ewes at 70–98 days gestation age (163–1065 g body weight). Blood samples, taken for gas analysis from an umbilical artery in nine of the younger group and from a carotid artery in seventeen of the older group, showed that the foetuses were in reasonable condition (Table 1).

In the twenty-two lambs of 40–55 days gestation tapping the nose or the face between the eye and mouth elicited a response as described by Barcroft (1946) consisting of a movement of the body, 'a sort of writhe', involving the head, trunk and limbs. Visual inspection often suggested that the intercostal muscles were involved in the response; but intrathoracic or tracheal pressure records showed that there was rarely more than a brief *rise* in pressure (as at the arrow in Fig. 2*a*). Only in two twin lambs of 40 days gestation was there a *fall* in intrapulmonary pressure, in ten of fifteen trials on tapping the face; there were also episodes of spontaneous activity in which intrathoracic pressure fell, in these as in other lambs at this age. Indeed the most striking feature in all lambs was the appearance of brief outbursts of spontaneous, irregular respiratory activity (Fig. 2). These were occasionally observed before delivery from the uterus, through the intact amnion. In nine lambs of 55 days gestation the spontaneous movements were present so often that it was not easy to make a fair test of the response to tapping the face. Gentle immobilization of the foetus reduced the incidence of spontaneous respiratory and other movements.

TABLE 1. Blood gas values in immature foetal lambs delivered into a warm saline bath under spinal or epidural anaesthesia

Gestation age (days)	Artery sampled	Number	P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)	pH
40–55	Umbilical	9	$21 \pm 1 \cdot 9$	$35 \pm 2 \cdot 1$	$7 \cdot 36 \pm 0 \cdot 03$
70–98	Carotid	17	$22 \pm 0 \cdot 9$	$40 \pm 1 \cdot 1$	$7 \cdot 37 \pm 0 \cdot 01$

Means $\pm$ S.E.

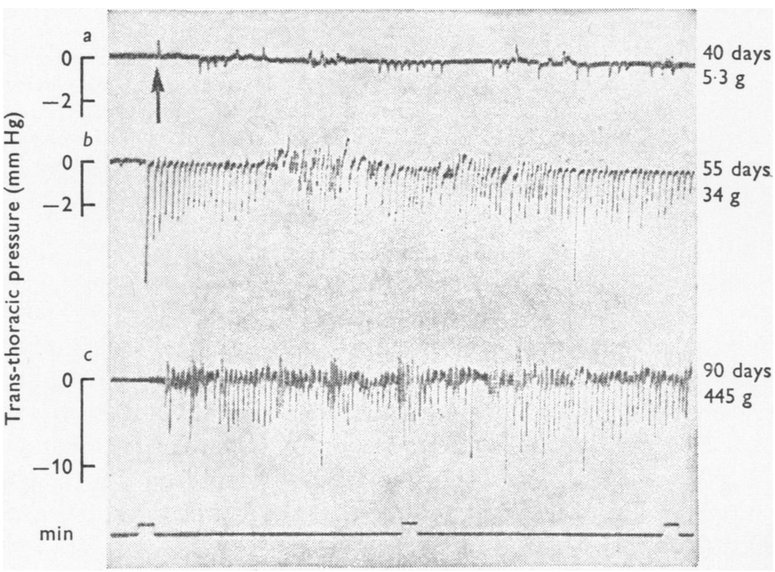

Fig. 2. Records of thoracic pressure changes during spontaneous respiratory movements in foetal lambs of different ages in a warm saline bath, recorded from catheters introduced through the chest wall (*a*) or into the trachea (*b*, *c*). At the arrow the face was gently tapped.

Spontaneous bursts of respiratory activity were also regularly present in the lambs of 70–98 days gestation as shown by movements of the chest wall accompanied by a fall in intrathoracic pressure recorded from tracheal, oesophageal and/or carotid catheters (as the foetal chest contains no air, intrathoracic pressure changes are transmitted to the systemic vasculature). With increasing age the size and frequency of respiratory movement during spontaneous activity rose from $2 \cdot 3 \pm 0 \cdot 3$ mm Hg and 53 ± 2 per minute at 45–55 days to $10{\cdot}4 \pm 1{\cdot}0$ mm Hg and 70 ± 7 per minute at 90–98 days (e.g. Fig. 2). With increasing age it became clearer also that there were two types of movement, single inspiratory efforts or bursts of rapid irregular activity. The absence or reappearance of activity did not bear any simple relation to the blood gas values (as between different lambs), or to the light or sound to which the foetus was exposed in the warm saline bath.

Chronic experiments on lambs in utero

Continuous observations were made on sixteen lambs for 7–14 days after implantation of catheters; they were then used in acute experiments or delivered by natural or induced labour. The series fully covered the last third of gestation. Observations were also made, for 2–6 days, on eleven lambs within the same age range, which subsequently succumbed as a result of infection, premature labour or other accidents. The results were similar in both groups, in that all twenty-seven lambs showed discontinuous respiratory activity of the same character.

Two types of respiratory activity were distinguished. The first, described in future as *gasping*, consisted of single brief relatively deep inspiratory efforts recurring irregularly at a slow rate (e.g. 1–3/min). The second, described as *rapid irregular breathing*, consisted of bursts of activity of very much higher frequency (1–4 Hz) lasting from a few seconds to an hour; the inspiratory movements were irregular in both rate and depth. Both are illustrated in Fig. 3, which shows a continuous tracheal pressure record over a $4\frac{1}{2}$ hr period in a lamb *in utero* whose mother was sitting undisturbed. The conclusion that the falls in intratracheal pressure in this and similar records were due to respiratory movements is supported by the fact that they were accompanied by falls in oesophageal pressure, with no change in amniotic fluid pressure. There were simultaneously, small forward-and-backward tracheal fluid flows (Fig. 4), as described below. Two lambs of 117 and 128 days gestation, whose tracheal pressures had already been recorded *in utero* for 8–11 days, were delivered into a warm saline bath under maternal epidural anaesthesia; movements of the chest and abdomen, characteristic of breathing, were observed coincident with changes in pressure recorded from the tracheal catheters (still *in situ*) similar to those *in utero*. The movements of the ribs were associated with a change in the shape of the chest.

Records of amniotic and tracheal pressures were made continuously, night and day, for many days after the implantation of catheters. The proportion of time, during which the two distinctive types of respiratory movement were certainly present, was estimated. In arriving at this estimate only clear records were used, for over 1400 hr recording time in thirteen lambs. Those records disregarded amounted to 7·6 % of the total on average. In most lambs there was little irregular rapid breathing during the first 24 hr after operation (Fig. 5). Thereafter there was a highly significant increase, so that by the third day breathing of one kind or another was present for at least a third of the time. There was no significant change in the proportion of each 24-hr period occupied by breathing after the third day from operation.

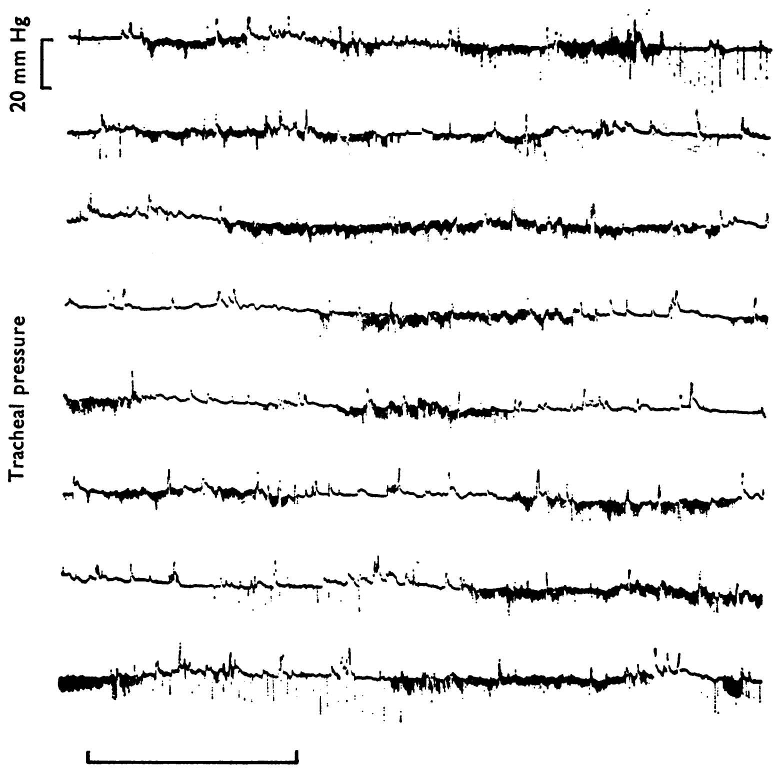

Fig. 3. Continuous tracheal pressure record over 4½ hr in a singleton foetal lamb *in utero*, 122 days gestation, 5 days after implantation of catheters. Carotid P_{O_2} 23 mm Hg, P_{CO_2} 51 mm Hg, pH 7·31. The lamb was in good condition when delivered into warm saline 3 days later.

Over the period of gestation studied (from 92 days until term) foetal respiratory movements became deeper, and the peak frequency of rapid irregular breathing increased, but there was no obvious change with age in their incidence or periodicity. The bursts of rapid irregular breathing showed no diurnal rhythm, and were unrelated to the environment (i.e. changes in illumination or sound). They were not excited or interrupted by the ewe standing up or sitting down. The respiratory movements of twins were unrelated even when, as in one instance, they were in the same uterine horn (but different amniotic sacs).

In foetal lambs there is a rapid increase in the size of the adrenal cortex during the last 10 days before term, attributed to ACTH secretion (Liggins, 1969). This is associated with a large rise in foetal plasma corticosteroid concentration (Bassett & Thorburn, 1969), which leads to the onset of parturition. Records were available from three ewes which went into labour at or near term. In none was there any significant change in rapid

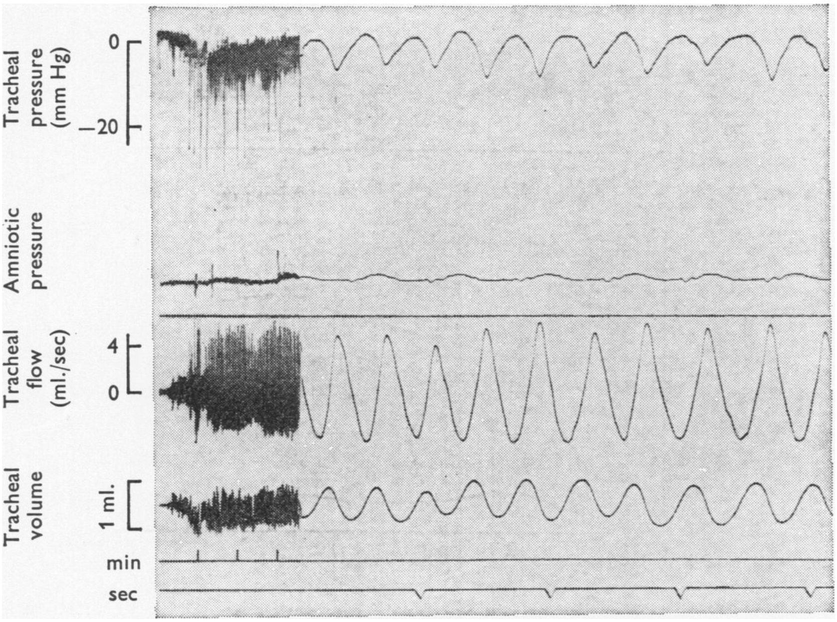

Fig. 4. Records from a lamb at 135 days gestation *in utero* of tracheal and amniotic pressures (recorded at the same manometer sensitivity) and of tracheal flow and fluid displacement from an electromagnetic flowmeter implanted just below the larynx.

irregular breathing before the onset of labour. So it seems unlikely that the naturally occurring ACTH and corticosteroid secretion directly modifies this type of respiratory activity. In only one of the three ewes was the record of foetal breathing movements *in utero* during labour of satisfactory quality. In this lamb there was a large reduction in such movements during labour.

Blood gas values. The mean carotid blood gas values of twenty foetuses *in utero* are shown in Table 2. The ewes soon became accustomed to our presence and the mild degree of maternal hypocapnia may be a normal concomitant of advanced pregnancy. There was no significant variation in the mean carotid P_{O_2} (Fig. 5) or P_{CO_2} after the first day from operation. However there were wide variations in blood gas values between lambs, and from time to time in the same lamb. Two days after operation the carotid P_{O_2} varied from 38 mm Hg at 104 days gestation to 9 mm Hg in a lamb of 127 days gestation (the correlation with age was not significant)

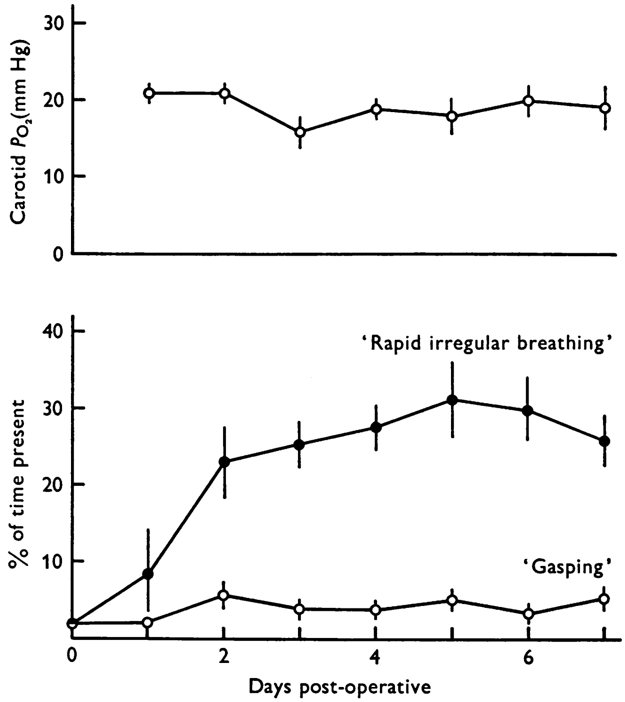

Fig. 5. The incidence of gasping and rapid irregular breathing in thirteen foetal lambs, of 108 days gestation to term, in each 24 hr period over the first 7 days after implantation of catheters (mean values ± S.E.)

TABLE 2. Mean ± S.E. carotid arterial blood gas values taken two days after implantation of catheters into twenty foetal lambs of 94–141 days gestation age

	P_{O_2}	P_{CO_2}	pH
Maternal	96 ± 3·5	33·2 ± 1·2	7·44 ± 0·01
Foetal	22 ± 1·5	47·5 ± 2·0	7·32 ± 0·01

and the P_{CO_2} from 40 to 66 mm Hg. In individual lambs (eleven to twenty-two samples over several days from each of ten lambs) the variations with time were large (P_{O_2} by 5–14 mm Hg, P_{CO_2} by 11–23 mm Hg). The variations in foetal carotid P_{CO_2} were significantly related to those in the mother, but the variations in P_{O_2} were not (e.g. Fig. 6). In one lamb only (the smaller of twins in the same horn studied between 123 and 132 days gestation) did the carotid P_{O_2} fall below 10 mm Hg (Fig. 7); samples were measured in duplicate. The P_{O_2} was less than 10 mm Hg on 3 days, with a maternal $P_{O_2} > 90$, and no evidence of progressive metabolic acidaemia (base excess 4 m-equiv/l.) or signs of cardiovascular distress in the foetus. The haematocrit (and hence the O_2 capacity of the blood) was exceptionally high, 53–59 %.

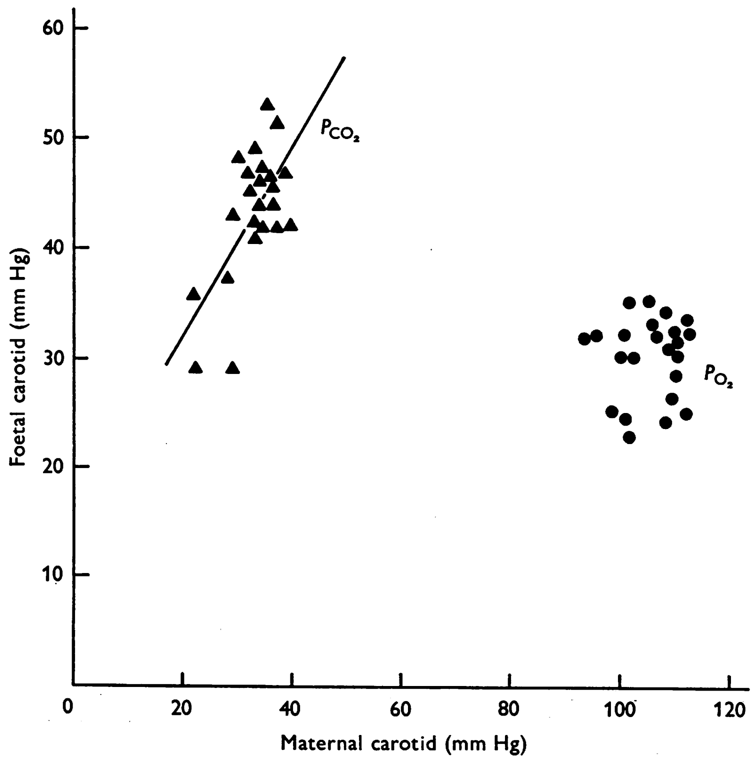

Fig. 6. Twenty-two observations on the relation between maternal and foetal carotid P_{O_2} and P_{CO_2} values over a 5-day period starting 2 days after implantation of catheters at 101 days gestation.

The presence or absence, frequency and size of foetal respiratory movements were unrelated to the blood gas values over the range of spontaneous variation encountered, either within a single lamb or between lambs, excluding only that in which the carotid $P_{O_2} < 10$ mm Hg. There was no significant difference between samples drawn repeatedly from five lambs in the presence or absence of movements. It was concluded that foetal respiratory activity is not normally determined or regulated through the activity of the central or peripheral chemoreceptors over the normal foetal physiological range of gas tensions.

In the one lamb in which carotid arterial P_{O_2} was periodically < 10 mm Hg, the incidence of rapid irregular breathing was less than either that of its twin, whose carotid P_{O_2} was greater (Fig. 7) or of other lambs of the same age (Fig. 5). The blood glucose concentration also was unusually low (6–12 mg/100 ml.) in this lamb. This single observation shows that there can be a lower level of foetal oxygenation, compatible with foetal survival but with a substantial reduction in the frequency of respiratory movement.

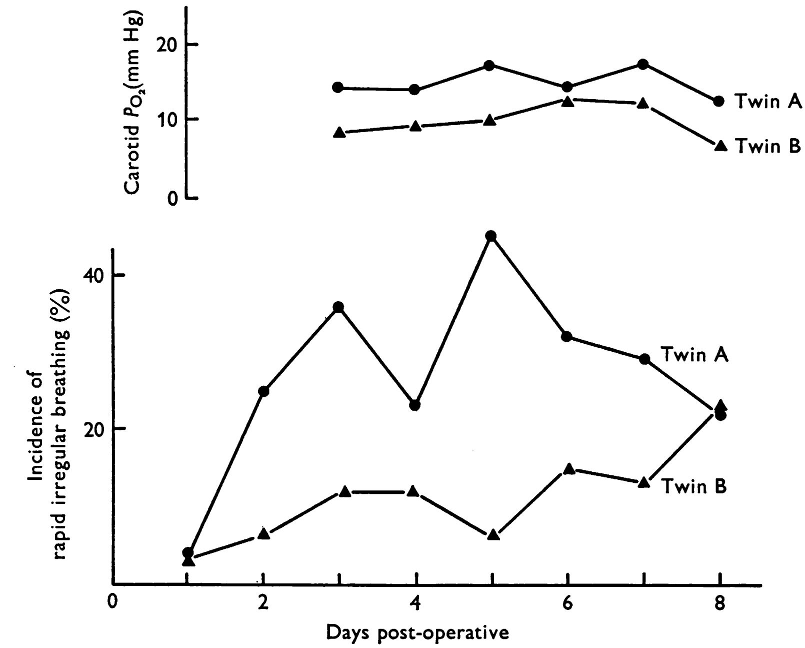

Fig. 7. Observations on twin lambs, of 123 days gestation at the time of implantation of catheters *in utero*, to show daily variations in carotid P_{O_2} and in the incidence of rapid irregular breathing.

Tracheal fluid movements. Fluid movement within the trachea was recorded for 7–13 days in five lambs of 125 days gestation onwards *in utero*. With each inspiratory effort, as seen by the fall in tracheal pressure, there was movement of fluid within the trachea. The peak tracheal flow rate was relatively high, up to 6 ml./sec, but the inspiratory movements were so brief ($< 0{\cdot}2$ sec) that the change in fluid volume rarely exceeded $\pm 0{\cdot}5$ ml. (Figs. 4, 8*c*). This is less than a tenth of the tracheal volume. Sometimes, in lambs within a week of term, the peak inspiratory efforts during rapid irregular foetal breathing were nearly -40 mm Hg, but so brief that the fluid volume displacement was still no greater.

When a foetal lamb is asphyxiated by temporary occlusion of the umbilical cord the gasping efforts are much greater (with falls of tracheal pressure to -75 mm Hg) and more prolonged (> 1 sec). In these circumstances larger quantities (> 10 ml.) of fluid (amniotic fluid with or without meconium) are inhaled into the tracheo-bronchial tree, even though the density and viscosity of this fluid are so much greater than those of air. This is consistent with the fact that amniotic debris and meconium may be seen emerging from the trachea of a foetal lamb during recovery from asphyxia, but are not normally present within the lung even though the lamb breathes *in utero*.

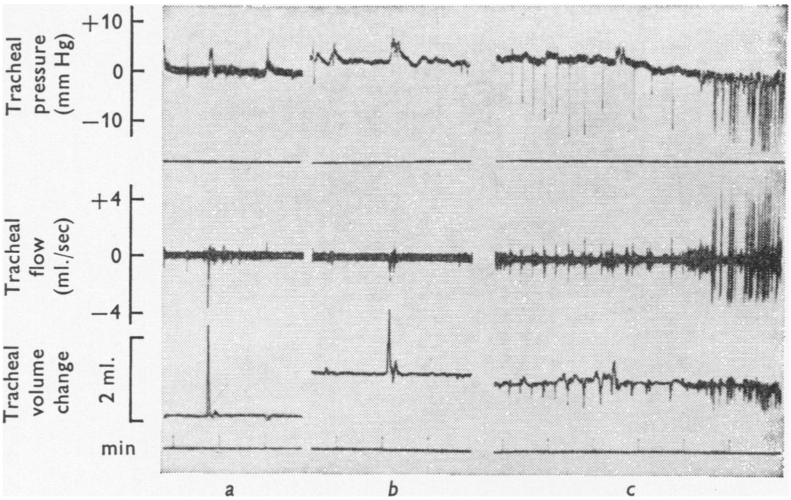

Fig. 8. Records from a lamb *in utero* of 134 days gestation, 4 days after implantation of catheters, to show examples of isolated expiratory efforts (*a*, *b*). Gasping and rapid irregular breathing are shown for comparison (*c*).

Inspection of the tracheal flow records in the five foetal lambs *in utero* also showed, from time to time, a movement of fluid of about 2 ml. volume outwards from the lungs associated with a brief rise in tracheal pressure, during a period when the foetus was not making breathing movements. Such incidents usually occurred once or twice an hour. Two examples are illustrated in Fig. 8 (*a*, *b*) together with a record showing gasping and rapid irregular breathing in the same lamb for comparison (*c*).

Observations were also made on mature lambs delivered under maternal epidural anaesthesia into a warm saline bath, either with or without tracheal and carotid catheters previously implanted. Rapid irregular breathing was associated with a very small movement of fluid in and out of the nostrils and mouth. This movement was made more readily visible by injecting small quantities of Indian ink around the muzzle.

Cardiovascular changes. Identical variations in amniotic fluid and foetal tracheal and arterial pressures were observed from time to time, attributed to maternal movements or to uterine contraction. Such variations have been taken into account.

The onset of rapid irregular breathing in the foetus was sometimes accompanied by a rise in heart rate, and often by a rise of blood pressure which, when the movements were most vigorous, was as much as 20%. Indeed the heart rate and blood pressure were noticeably irregular from time to time in all lambs.

Records of descending aortic blood flow (below the origin of the renal arteries) were obtained in three lambs from 122 days gestation onwards, from 3 to 7 days after implantation of flowmeters into the aorta and

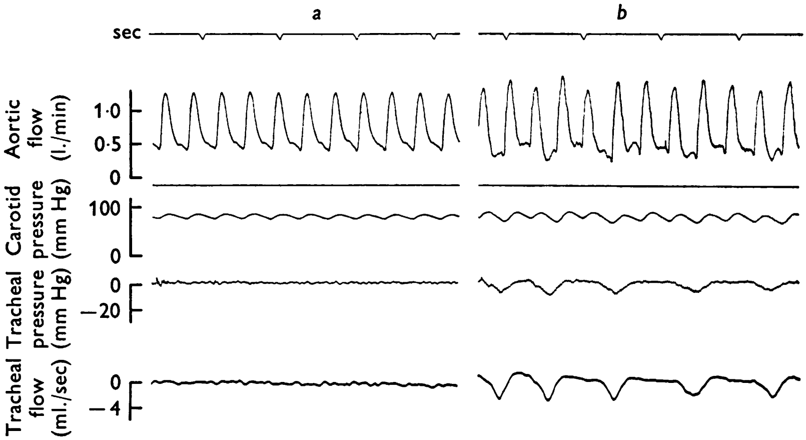

Fig. 9. Record from a lamb *in utero* of about 130 days gestation, 4 days after operation, to show the variations in flow and pressure in the absence (*a*) and presence (*b*) of rapid irregular breathing.

trachea (after which the ewes went into labour). The aortic flow records differed from those in animals after birth (e.g. adult dogs) in that forward flow remained well above zero in diastole (Fig. 9). Most of the flow must have gone through the umbilical cord to the placenta (placental flow is about half the combined output of both ventricles), and indeed tying the cord on delivery of one foetus caused an abrupt fall in aortic flow to less than one-tenth of the previous value.

Normally phasic aortic flow records were regular (Fig. 9*a*), but when the foetus started breathing the brief falls in arterial pressure (with each decrease in intrathoracic pressure) were accompanied by commensurate changes in aortic flow, which became noticeably irregular (Fig. 9*b*). This irregularity was also detectable on smoothed records (time constant 1·2 sec). Mean aortic flow showed additional types of short term and long term variations. For instance, in the lamb, part of whose record is shown in

Fig. 9, mean flow was normally about 650 ml./min (180 ml. $kg^{-1}.min^{-1}$) but showed brief falls to less than 400 ml./min and rises to more than 900 ml./min within 30 min. Longer lasting changes of smaller size (e.g. between 560 and 750 ml./min over 2 hr) also were seen.

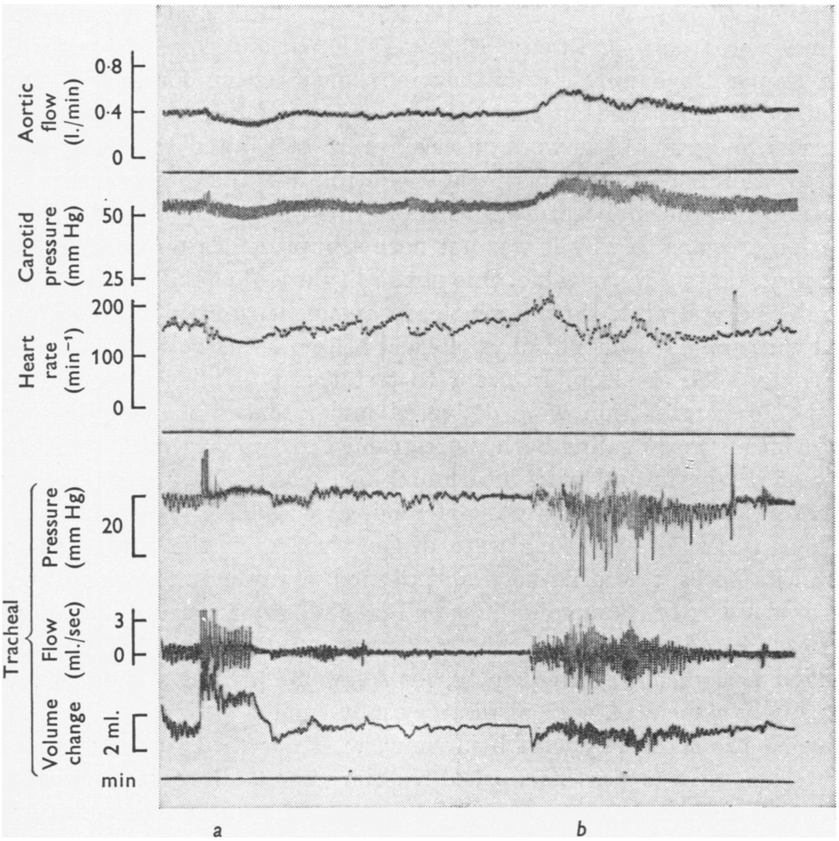

Fig. 10. Record from a lamb *in utero* of 126 days gestation, 4 days after operation to show cardiovascular changes associated with expiratory efforts (*a*) and the onset of unusually vigorous breathing movements (*b*).

The short term variations in aortic flow were almost always correlated with respiratory movements. Fig. 10*a*, *b* shows good examples. At *a* the foetus made three expiratory efforts, as recorded on the tracheal pressure record, with expulsion of nearly 3 ml. fluid. This was accompanied by a relatively large fall in heart rate, in carotid pressure and in aortic flow from 400 to less than 300 ml./min. Conversely at *b* the onset of rapid irregular breathing was accompanied by tachycardia, a rise in carotid pressure and in aortic flow to 580 ml./min. Thus respiratory movements of different kinds were accompanied by relatively large cardiovascular changes. These complex patterns were seen repeatedly in continuous records day by day.

Rapid-eye-movement sleep. When mature foetal lambs are delivered under maternal epidural, spinal or local anaesthesia and are gently restrained on a warm table, with intact umbilical cords, they do not normally breathe (e.g. Dawes, 1968, p. 131). In only five of twenty-five such preparations were respiratory movements seen, for only 5–15 sec out of an hour. At the time that these observations were made, some years ago, such movements as were observed were attributed to casual tactile or cold stimuli beyond experimental control. With the recognition that breathing is a normal feature of intrauterine life in the foetal lamb, the question arose as to why it was not normally present after delivery from the uterus on to a warm table. One possibility was that the unusual tactile stimuli and restraint, though gentle, in an unaccustomed position interfered with some features of physiological behaviour associated with foetal breathing, such as sleep. In order to test this possibility, ten lambs of 115–147 days gestation were delivered under maternal epidural anaesthesia into a warm saline bath, unrestrained except by contact with the walls of the bath, and with intact umbilical cords. They were observed for many hours, with as little interference as possible.

Three types of behaviour were distinguished. In the first the lamb moved its limbs, raised its head and opened its eyes. In this condition it appeared aware of its surroundings and reacted vigorously and at once to tactile and auditory stimuli. In fact it appeared awake. This condition recurred periodically, infrequently, for a few minutes at a time and was not usually associated with respiratory movements.

During the second type of behaviour, which seemed to correspond to quiet sleep and was present most of the time, the lamb reacted sluggishly to stimuli and there were usually no movements apart from occasional protrusion of the tongue, swallowing and slow, gentle extensions of the limbs. In this state the lamb's head and limbs seemed to float in the bath; there appeared to be little muscular tone even in the neck.

The third type of behaviour appeared to be of rapid-eye-movement sleep. It was accompanied by bursts of rapid irregular respiratory movements identical in character to those recorded *in utero*, but often shorter in duration and less frequent. They ceased as soon as the foetus was handled. Their appearance was associated with opening and rapid movements of the eyes (after about 125 days when the lids become separated), which was not seen otherwise. Brief intermittent twitches of the ears, mouth and limbs were seen simultaneously. Delivery of the lamb from the warm saline bath on to a warm table, with gentle restraint, arrested respiratory and rapid-eye-movements for an hour; both returned a short time after the lamb was immersed once more.

In two foetal lambs near term records were made for 7–10 days after implantation of electrodes for recording eye movements (and the electrocorticogram; see below) as well as tracheal, amniotic and carotid catheters. Rapid irregular breathing was observed only in association with a large increase in eye movement activity (Fig. 11).

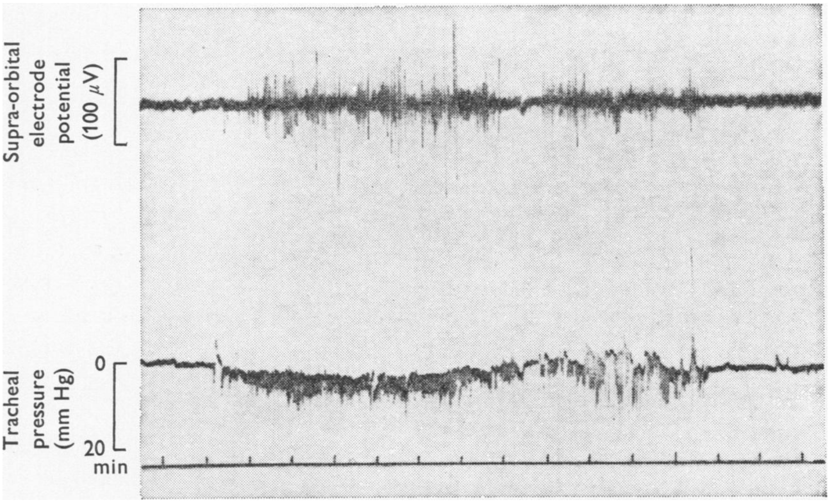

Fig. 11. Record from a lamb *in utero* of about 130 days gestation, 6 days after operation, to show the association of eye movements (recorded from supra-orbital electrodes) with rapid irregular breathing.

The electrocorticogram was recorded *in utero* from nine lambs of 110–138 days gestation age. In five of these the recording was almost continuous over 7–14 days. Two types of activity were observed from biparietal dural electrodes. The most frequent was one of predominantly fast (10–20 Hz) low voltage ($<$ 25 μV peak) activity (Fig. 12*a*), present 54–69 % of the time as calculated from 24 hr periods of observation. Otherwise the activity was predominantly slow (3–12 Hz) and of high voltage (40–100 μV peak; Fig. 12*b*). The presence of one or other type of electrocortical activity seemed to bear no relation to the duration of time from operation. In lambs of less than 128 days gestation it was difficult to distinguish the two types of activity at a low paper speed (9 mm/min) because the peak voltage was only 20–40 μV.

Rapid irregular respiratory movements were always associated with predominantly fast low voltage electrocortical activity, but they were not invariably present when the activity was of this character. For instance, during the first 2 days after implantation of the electrodes and catheters respiratory movements were very infrequent (< 10% of the time), though fast low voltage electrocortical activity was present more than half the time. The change in character of the electrocorticogram (from slow high voltage to fast low voltage) always preceded the onset of irregular rhythmic respiratory movements, and the latter always ceased

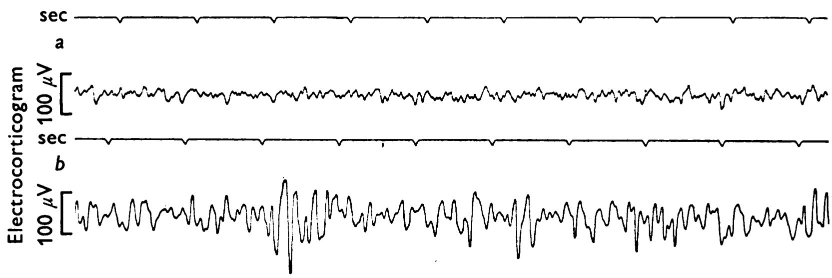

Fig. 12. Records of the biparietal dural electrocorticogram in a lamb *in utero* of 143 days gestation 5 days after operation, showing predominantly low voltage rapid activity (*a*) and high voltage slow activity (*b*, as in quiet sleep).

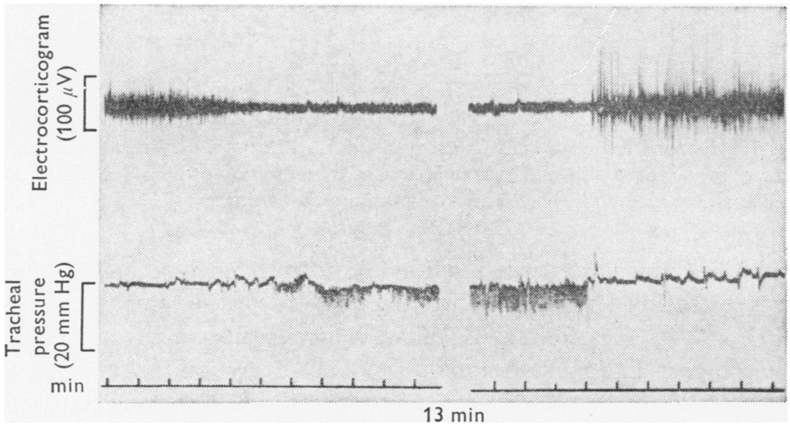

Fig. 13. Records from the same lamb as in Fig. 12, to show the association of rapid irregular breathing with predominantly low voltage rapid electrocortical activity.

before the reversal of electrocortical activity (Fig. 13). These observations are consistent with the conclusion that this type of respiratory behaviour is associated with a form of rapid-eye-movement sleep. Gasping was not associated with any particular electrocortical activity.

Interruption of the cervical vago-sympathetic trunk by section or local anaesthesia. Interruption of nerve conduction in the vagi by section (at operation for insertion of the foetal catheters) or by local anaesthesia (applied *in utero* through fine catheters already implanted) did not modify the size, frequency or incidence of rapid irregular breathing movements. The efficacy of vagal local anaesthetic block was tested after delivery of the lamb under maternal chloralose anaesthesia, the catheters remaining *in situ*. Injection of the same anaesthetic solution (xylocaine 1 % (w/v) in 0·9 % saline, 0·2 ml. bilaterally) blocked the femoral vasoconstriction associated with foetal hypoxaemia (which is dependent upon the integrity of the aortic nerves; Dawes *et al.* 1969) and also the bradycardia caused by electrical stimulation of the peripheral ends of the vagi, cut higher in the neck.

These observations are interesting for two reasons. First, they demonstrate that the respiratory movements normally present *in utero* are independent of the Hering–Breuer reflexes. These can be elicited in foetal lambs by changing the lung volume (Dawes, 1968, p. 132). The fact that the normal movements are independent of the pulmonary stretch reflexes is consistent with the conclusion that they are not associated with inhalation of any substantial volume of fluid. Secondly, vagal section or block interrupts conduction in afferent nerves from the aortic bodies and other types of sensory endings in the heart, lungs and great vessels, and also the efferent cervical sympathetic nerves running in the mid-cervical vagal trunk to the carotid bodies. Purves & Biscoe (1966) suggested that increased activity in the latter might be one factor accounting for a change in carotid body activity when the cord is tied after delivery. Evidently neither of these systems normally modifies rapid irregular respiratory movements in the foetus.

General anaesthesia. When the maternal abdomen is opened under light general anaesthesia, respiratory movements are almost never seen in foetal lambs observed through the uterine wall or amniotic sac, even when the sac is intact and the lamb is undisturbed. It seemed possible that general anaesthetics, in comparatively small dosage, might arrest foetal respiratory movements.

One ewe was given chloralose 15 mg/kg I.V., a dose which caused only sedation; the corneal reflex was still present. Foetal respiratory activity, which had been observed for many hours, ceased at once and did not return over a period of more than an hour. Similarly administration of pentobarbitone, only 15 mg/kg, I.V. to another ewe caused arrest of foetal respiratory movements for nearly an hour.

DISCUSSION

Changes in foetal tracheal and oesophageal pressures similar to those described as rapid irregular breathing (Dawes *et al.* 1970) were reported at the same time by Merlet, Hoerter, Devilleneuve & Tchobroutsky (1970) in eight lambs of 113–133 days gestation using the same method for implanting catheters of the same dimensions. They too found no apparent relationship between the blood gas values and the onset or cessation of respiratory movements. The character of the rapid irregular respiratory movements of the foetus *in utero*, their very irregularity, evanescence, susceptibility to general anaesthesia, lack of correlation with carotid arterial blood gas values over a wide range, and the relatively small changes in thoracic volume (compared with those after birth) go far to explain why there have been such conflicting reports in the past.

In recent years it has been concluded that foetal breathing is not normally present *in utero* for three reasons. First, because Barcroft (1946) had described in foetal lambs after 60 days gestation an inhibition of the allegedly normal response of the respiratory musculature to tactile stimuli. Yet, as we have seen, respiratory movements occur spontaneously both before and after this age, when studied under similar experimental conditions in warm saline. Secondly, injection of radio-opaque contrast medium into the amniotic fluid in women (McLain, 1964) and animals (Carter, Becker, King & Barry, 1964; King & Becker, 1964) led to its appearance soon afterwards in the gastro-intestinal tract, but not the lungs. Yet vigorous foetal breathing movements are compatible with comparatively small changes in lung volume. Insufficient contrast medium was inhaled to delineate the trachea and bronchi; also the contrast medium must have been diluted with pulmonary fluid, which is produced continuously. Thirdly, breathing movements are not normally seen in foetuses exteriorized with intact umbilical cords, on to a warm table adjacent to the ewe, even when no general anaesthetic has been used. Nor are they regularly present in foetal lambs either retained within or returned to the uterus soon after operative interference. These conditions are apparently incompatible with normal intrauterine behaviour, though respiratory movements are regularly seen after delivery into a warm saline bath, provided there is minimal restraint and neither foetal incisions nor general anaesthesia.

Direct observations were made of the respiratory movements of foetal lambs delivered in warm saline. These showed that the tracheal pressure records of rapid irregular breathing were associated with vigorous movements of the diaphragm and rib cage and striking changes in the shape of the chest. These were not hiccups, such as have been invoked, without direct evidence, to account for some foetal movements. The rapid irregular breathing and single brief deeper inspiratory efforts (described as gasps) were not associated with movements of the limbs, head, neck or spinal column. The movements were confined to the respiratory musculature alone. They are not a casual association with general movements of the foetal body.

All the respiratory movements, both inspiratory and expiratory, were accompanied by flow of tracheal fluid in the appropriate direction. *In utero* the amount of fluid moved from time to time (e.g. Figs. 8 and 10) is clearly incompatible with closure of the glottis. Even the relatively small quantity of fluid moved during rapid irregular breathing (Fig. 4) suggests that the glottis is not shut, since the tracheal flowmeter was placed only just below the larynx. Nevertheless, partial closure of the glottis might cause an alteration in the size of the tracheal pressure changes. In lambs delivered with intact umbilical cord into a warm saline bath it was often possible to see small movements of fluid in and out of the nostrils, associated with breathing movements. This could well explain how it comes about towards term that traces of lung surfactant appear in the amniotic fluid. Otherwise it has been supposed that such pulmonary secretions were swallowed.

It is very unlikely that the undoubted respiratory movements observed are artifacts resulting from experimental interference. First, they are present in all lambs, in good physiological conditions as judged by their blood gases. Some of these lambs were delivered naturally, alive and well at or near term, after up to 2 weeks observation *in utero*. Secondly, identical respiratory movements were observed in foetal lambs delivered into a warm saline bath, whether catheters had been implanted 1 or 2 weeks previously, or whether no operative interference had been undertaken other than delivery. And thirdly, during operations on sheep under epidural anaesthesia, movements were sometimes seen in foetal lambs through the intact uterine wall, which appeared to be respiratory in character. They continued, and were certainly identified as rapid irregular breathing, after incision of the uterus so as to permit inspection through the intact amnion.

Thus these movements cannot be attributed either to partial asphyxia or to sensory stimuli arising from tracheal or oesophageal catheters. They also persisted unchanged after division or block of the cervical vagi, and hence of the principal afferent nerves from the trachea, bronchial tree and oesophagus. This excludes the possibility that the movements resulted from the casual irritation of cough receptors, for instance, among other types of sensory endings within the trachea and carina.

Our knowledge of the wide range of respiratory movements made by the foetal lamb *in utero* is not yet sufficiently precise to identify the physiological character of the various patterns which can be distinguished. The occasional expiratory efforts (e.g. in Figs. 8*a*, *b* and Fig. 10*a*) might be coughs, grunts or bleating. From time to time deep inspiratory and expiratory efforts suggestive of sighing, yawning or sneezing have been observed. The much more common phenomenon described as rapid irregular breathing might be panting, recurring as it does at irregular time intervals, with a frequency similar to that in adult sheep (e.g. Bligh, 1959), in foetuses immersed in amniotic fluid at a temperature of nearly 40° C.

It remains to be seen whether foetal breathing movements are present in other species. Previous observations on animals, and largely anecdotal observations on man, summarized, for instance, by Windle (1941), suggests that they may well be. B. Leduc & J. S. Robinson (unpublished) have recently recorded such movements in foetal rabbits during the last 3 days of gestation.

One of the peculiar features of irregular rapid breathing is its very irregularity in rate and depth. Shortly after birth, even premature birth, regular rhythmic breathing is soon established, interrupted only by a sigh at irregular intervals. In foetal lambs during the last third of gestation, delivered from the uterus on to a warm table under maternal spinal or general anaesthesia (and hence not normally making respiratory movements), it is possible to induce gentle breathing, regular both in rate and depth, by application of cold (Dawes, 1968) or by raising the foetal arterial P_{CO_2} above 60 mm Hg, while the P_{O_2} is 20–25 mm Hg; the trachea and lungs were still filled with fluid. Hence the irregularity cannot be attributed to immaturity or to other features of the foetal condition. The fact that respiratory movements are noticeably irregular during rapid-eye-movement sleep in new-born human infants is relevant.

Rapid-eye-movement sleep. The most convincing evidence of the existence of varying phases of sleep and wakefulness in the foetal lamb is derived from observations in a warm saline bath. This makes it possible to study the reactions of the lamb to sensory stimuli and to observe spontaneous movements of its eyes, nose, mouth, trunk and limbs. Such observations suggest that the foetal lamb normally has little tone in its skeletal, including its cervical, musculature. In quiet sleep (as indicated by its sluggish response to stimuli and the absence of eye and other characteristic movements) the head appears to float freely. Ruckebusch (1971) has concluded from electromyographic records of foetal lambs *in utero* that there is normally little tone in the neck muscles, even during what he concludes, using somewhat different criteria, is quiet sleep. We must accept that cervical muscular tone, which is regarded as a necessary concomitant of quiet sleep after birth, disappearing during rapid-eye-movement sleep, is not normally present before birth when the foetus is suspended in a warm fluid. Its presence after birth must be a consequence of the extrauterine environment; and its utility as a diagnostic criterion of the difference between quiet and rapid-eye-movement sleep is probably limited to life after birth.

The correlation of rapid irregular breathing with the phenomena usually attributed to rapid-eye-movement sleep (eye movements, the character of the electrocorticogram and the other movements seen in a warm saline bath) so far has been made only in mature foetal lambs, from 115 days gestation onwards. The proportion of time occupied by rapid irregular breathing in the present experiments after the first 2 days from operation (Fig. 5) corresponds with the time calculated to be occupied by rapid-eye-movement sleep (40 %) by Ruckebusch (1971) in foetal lambs near term. At gestational ages earlier than this it may be difficult to distinguish the characteristic changes in electrocortical pattern. Bursts of rapid irregular breathing and gasping activity were discontinuously present in lambs from 40 days gestation onwards. In this respect our conclusions differ from those of Barcroft & Barron (1937*a*). They were inclined to attribute such respiratory movements as were visible to the effect of external sensory stimuli. We found that in foetuses of 40–55 days gestation it was impossible to be sure that a movement of the thoracic musculature was associated with an inspiratory effort unless there was a record of intrathoracic pressure available. Using such records, tactile stimulation of the nose or face was rarely associated with an inspiratory effort, or succession of efforts. On the contrary, these appeared spontaneously at erratic intervals and it is tempting to suppose that they may of the same origin as those seen later in gestation, after 115 days from conception.

The first spontaneous electrical signs of cortical activity do not appear until about the 60th day of gestation in foetal lambs (Bernhard, Kaiser & Kolmodin, 1959; Bernhard & Meyerson, 1968), and at this age originate from subcortical areas. But in the preparations which Bernhard and his colleagues have studied, respiratory movements were rarely present (personal communication). So even at this early age, activity in the respiratory centre may be inhibited by the restraint and dissection necessary to approach the cerebral cortex. This would be consistent with the early maturation of the respiratory centre, as compared with the cerebral cortex in which inhibition of spontaneously discharging units did not occur after peripheral sensory stimulation until much later, more than 80–90 days (Meyerson & Persson, 1969). We conclude that the episodic activity of the foetal respiratory centre early in gestation, from 40 to 55 days, arises spontaneously; the nature of the subsequent association with eye movements and the relatively low voltage rapid electrocortical activity remains to be explored.

Cardiovascular and respiratory variations. The results show that the conditions of foetal life *in utero* are highly variable, from time to time in the same lamb, in respect of respiratory movements (Fig. 3), carotid arterial blood gas values (Fig. 6) and the cardio-vascular system (Fig. 10). Thus single measurements of blood gas values, or of cardiac output and its distribution, may give a misleading impression, especially when samples are withdrawn or flow measurements made (e.g. using the isotope-labelled microsphere method) over a short period of time.

Also, the results show that returning the mature foetus to the uterus, after exteriorization for purposes such as the implantation of catheters, does not guarantee an immediate return of normality. It can take as long as 2–3 days for the re-establishment of normal intrauterine respiratory behaviour. Surgical intervention can also lead to the initiation of parturition, after an interval usually of 5–7 days, with rapid growth of the foetal adrenal (Liggins, 1969) and a swift rise in foetal plasma corticosteroid concentration (Bassett & Thorburn, 1969). But these phenomena do not appear to be related to foetal breathing, since rapid irregular breathing continues unaltered until the onset of labour.

The exceptional observations illustrated in Fig. 7 showed that it is possible for a foetal lamb to survive several days with a carotid arterial P_{O_2} of 10 mm Hg or less (but having a high haematocrit) and with no breathing movements for many hours at a time. This raises some interesting possibilities, as to whether foetal breathing *in utero* could be used as an index of normal foetal health, and whether respiratory musculature which is not regularly exercised *in utero* will tire soon after birth. It also suggests that there may be a degree of foetal hypoxaemia at which normal respiratory movements (rapid irregular breathing) are inhibited, but the gross inspiratory efforts associated with partial or complete asphyxia are not excited.

Incidentally, the results are inconsistent with normal function of the carotid bodies in foetal life, at least over the physiological range as known from observations in the adult. This conclusion agrees with other evidence from recording carotid nerve activity (Purves & Biscoe, 1966) and studies on the cardiovascular reflex effects of hypoxaemia (Dawes *et al.* 1969).

Finally, the normal but intermittent presence of foetal respiratory movements *in utero* could help to explain some of the apparently contradictory observations at birth, where some lambs and human infants are born breathing while others are not, irrespective of their blood gas values.

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